WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTÉ

WHO/Mal/189
WHO/Insecticides/63
26 June 1957

ORIGINAL: ENGLISH

ASSESSMENT OF SUSCEPTIBILITY TO INSECTICIDES IN ANOPHELINE MOSQUITOS

Summary of information received by the Malaria Section

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The Expert Committee on Malaria recommended in its Fifth Report (1954) the use of the Busvine and Nash method for assessing the susceptibility of anophelines to insecticides. In order to implement this recommendation, the Malaria Section has been distributing test outfits, consisting of standard insecticide solutions and necessary equipment, to entomologists and malariologists interested in the determination of the susceptibility to insecticides of anopheline mosquitos. The Malaria Section has received a considerable amount of information on the results of the tests carried out by the recipients of the test outfits and by other investigators. This information is summarized in the present note.

Results of susceptibility tests with anopheline mosquitos have been published in a certain number of cases, particularly when resistance to insecticides was suspected, and a very valuable survey of measurements of susceptibility in mosquitos was made recently by Busvine (1956), but the ever-increasing use of chlorinated hydrocarbon insecticides in malaria campaigns throughout the world and the danger of resistance developing among malaria vectors makes it necessary to give a wide circulation to all the information available on susceptibility tests carried out with anophelines.

The tests summarized in this note were carried out following the Busvine and Nash method (1953, 1954). They were, with few exceptions, carried out in the field; in most cases, engorged wild-caught females were used. Exceptionally, females bred in the laboratory from larvae captured in the field were tested, and in a few cases - which are indicated in the tables - the susceptibility tests were carried out with females from laboratory colonies. Tests involving a total of 35 different strains, corresponding to 16 different anopheline species - all of them malaria vectors - are summarized in the three tables included in this note. In Table I the tests made with DDT are summarized, in Table II, the dieldrin tests, and in Table III the tests carried out with gamma BHC.

It has been thought advisable to show in the above-mentioned tables the number of mosquitos tested with each insecticide concentration, the number of dead and the corresponding mortalities, and this has been done also with the controls. The reader can thus judge for himself the adequacy of the samples tested and the homogeneity of the results obtained with each different strain. The tables also give the median lethal concentrations (or LC_{50}'s) corresponding to each trial. They have been calculated on the corrected mortalities (adjusted after Abbott's formula) from which log-concentration/probit regression lines have been drawn and the LC_{50} has been graphically estimated except in a few cases in which the more precise method of numerical estimation has been followed. It will be noticed that in the three tables included in this report no distinction has been made between the susceptible and resistant strains. This is due to the fact that in this series of observations it would be difficult to draw a line between susceptible and resistant strains. Strains from treated areas often have an LC_{50} higher than the LC_{50} obtained with strains from unsprayed areas, but it would be difficult to call a strain from a treated area resistant unless its LC_{50} has become many times greater than the LC_{50} obtained in an unsprayed area. In most cases the small increase in the LC_{50} brought about by insecticide pressure could represent that small amount of tolerance (e.g. x2 to x10) which is inevitable when a population is submitted to any selective agent and which Hoskins and Gordon (1956) have termed "vigour tolerance" rather than the presence of a true resistant character.

It is interesting to note that, as can be judged by the LC_{50} values, there is not much variation in the susceptibility level of the anopheline strains from untreated areas. This is particularly noticeable in the case of DDT where a good number of trials have been carried out. With few exceptions in the parts of the world covered by these tests, the DDT LC_{50} for anopheline mosquitos not subject to insecticide pressure varies between 0.5 and 1.5%. The evidence presented in this summary may be considered as sufficient indication that these figures do in fact represent normal limits. LC_{50}'s above this range might then be taken as due to insecticide pressure representing either vigour tolerance or the presence of a true resistant character, even in the absence of comparable pre-treatment data. In this series of observations the following records of DDT LC_{50}'s below 0.5% were found: A. leucosphyrus leucosphyrus and A. leucosphyrus balabacensis - two closely related forms of the same complex - A. superpictus and A. sundaicus. The number of leucosphyrus females examined in both Sarawak and North Borneo is large enough and the conditions of the tests satisfactory, so that it must be accepted that the two forms of leucosphyrus examined in Borneo are more susceptible to DDT than other anophelines.

Regarding the low LC_{50} of A. superpictus obtained in Greece, it will be observed in Table I that the sample examined was comparatively small, but the results from other investigators (Ansari et al. 1956, Gramiccia et al. 1955 and Zulueta et al. 1957), who found an A. superpictus DDT LC_{50} of 0.75, 0.72 and 0.6% respectively, seem to indicate that this species has a comparatively low LC_{50} and that values below 0.5% could be expected.

The case of A. sundaicus is a similar one. There is an LC_{50} of 0.31% obtained in Burma which seems rather low for an anopheline species, but tests carried out on a very large scale in Java indicate that the LC_{50} of this species in an unsprayed area is 0.5%. Davidson (1955) also found in Java a sundaicus LC_{50} of 0.5%, so that this species seems to be more susceptible to DDT than the average anopheline.

The effect of insecticide pressure is, as pointed out before, evident in most cases. The strains from untreated areas usually have a higher LC_{50} than the strains from unsprayed areas. This, as Table I will show, is clear in the case of A. hyrcanus sinensis from Taiwan, A. gambiae from the Belgian Congo and A. leucosphyrus balabacensis from North Borneo where DDT LC_{50}'s obtained in treated areas are higher than those obtained in unsprayed areas. These should probably be attributed to an increase of "vigour tolerance" rather than the presence of true resistance.

The results obtained in Greece by Dr Hadjinicolaou are particularly interesting. In the area of Skala in southern Peloponnesus, susceptibility tests carried out by Livadas and Thymakis in 1954 and by Busvine in 1955 (Busvine, 1955) gave a DDT LC_{50} for A sacharovi of 2.6% and >3% respectively, which indicated that under sustained insecticide pressure resistance was on the increase. The results obtained in Asterion, Skala, in August 1956 indicate that a very marked degree of resistance to DDT has developed in A. sacharovi in the area. The LC_{50} has been numerically estimated in this case by Mr K. Uemura of the Statistical Section. As would be expected, a graphical estimation has little value in this case when the median lethal concentration is so far beyond actual concentrations used in the test. The numerically estimated LC_{50} is 122.0% and, according to Mr Uemura, this LC_{50} has the 95% confidence limits of 12 and 1250%. It seems, therefore, that the prolonged use of DDT and related chlorinated hydrocarbons has produced a very markedly resistant A sacharovi in the area of Skala in southern Greece.

The findings of Hadjinicolaou from other parts of Greece are more difficult to interpret. A DDT LC_{50} of 3% (Anthili, Lamia) and 2.7% (Nea Komi, Kavalla) in A. sacharovi are above the normal LC_{50} for this species. Whether this should be considered as "vigour tolerance" or as the beginning of development of resistance, it is difficult to say. In the Skala area the early findings could well fit into the "vigour tolerance" concept and yet 1956's results show that true resistance was in fact developing. A difficulty in the interpretation of the results from Greece has been the lack of information on the susceptibility level of A. sacharovi from untreated areas, which apparently cannot be found at present in Greece. There is mention in the literature of a sacharovi DDT LC_{50} of 0.2%, which has been compared with the LC_{50}'s obtained in Greece and elsewhere, but this median lethal concentration is considered an improbable estimate since an LC_{50} of 1.1% has been obtained with A. sacharovi from an untreated area in Iran (Zulueta et al. 1957) and since all the observations made with other forms of the maculipennis group from untreated areas have always given much higher LC_{50}'s. If the LC_{50} of 1.1% is taken as a basis for comparison, it will be seen that the results from Anthili, Lamia and Nea Komi, Kavalla, indicate a small increase of tolerance whereas the findings from Skala show a very marked degree of resistance in A. sacharovi.

Regarding the DDT LC_{50} of 1.2% obtained with A. maculipennis typicus in Poros (Greece), this should be considered as normal since the LC_{50}'s obtained with susceptible strains of this species have always been close to the above-mentioned figure. It may be mentioned here that the increased maculipennis DDT LC_{50} obtained in Podgorii, Romania, in September-October 1956 was probably due to the condition of the mosquitos, which, as the Romanian investigators point out, had developed fat body It should also be kept in mind that the observations made in Mexico with A. quadrimaculatus, A. aztecus and A. albimanus which gave comparatively high LC_{50}'s were made in September and October when the temperature was "cool, almost cold during the night", according to the report of Duret. Zulueta et al. (1957a) have shown that there was a great increase in the LC_{50} in A. maculipennis in Iran at the beginning of hibernation.

Before concluding these comments, we would like to point out the interesting dieldrin results obtained with A. gambiae (Table II) in the area of Ruzizi in the Belgian Congo. The use of DDT has apparently brought about an increased tolerance to dieldrin As Table II will show, a considerable number of females survived exposure to dieldrin concentrations of 0.4 to 1% dieldrin, but the true significance of this finding is not clear. In the experiments of Davidson (1956) the survival from dieldrin concentration of approximately 0.4% was an indication that the surviving mosquito was a hybrid of a resistant gambiae, but, whether this is the case in the findings of Ruzizi or whether they are due simply to increased "vigour tolerance", it is difficult to say.

In the case of Zanzibar, there were no survivals from a dieldrin 0.25% concentration and it is curious to observe that in the area of Ilaro in southern Nigeria treated repeatedly with BHC, there is no indication of a development of dieldrin-BHC resistance as observed in Western Sokoto in northern Nigeria.

REFERENCES

Ansari, N., Baghdiantz, A. & Samimi, B., (1956) Unpublished report, Malaria Institute, Teheran

Busvine, J. R. (1955) Unpublished report to WHO

Busvine, J. R. (1956) Bull. Wld Hlth Org. 15, 787

Busvine, J. R. & Nash, R. (1953) Bull. ent. Res. 44, 371

Busvine, J. R. & Nash, R. (1954) Wld Hlth Org. techn. Rep. Ser. 80, 30

Davidson, G. (1955) Unpublished report to WHO

Davidson, G. (1956) Nature, 178, 863

Gramiccia, G., Stevens, P. A., Faglich, M., Janbakhsh, B. & Arjomand, F. (1955) Unpublished report of the Arthropod-borne Diseases Control Project, Teheran

Hoskins, W. M. & Gordon, H. T. (1956) Annual Review of Entomology, 1, 89

Zulueta, J. de, Jolivet, P., Thymakis, K. & Caprari, P. (1957) WHO/Mal document No. 188

Zulueta, J. de, Jolivet, P., Thymakis, K. & Caprari, P. (1957a) Bull. Wld Hlth Org. 16, 475

TABLE I. SUSCEPTIBILITY TESTS BY BUSVINE METHOD - DDT RESULTS

Species	Investigator	Country	Locality or area	Sprayed or unsprayed	Date	DDT concentration in the non-volatile oil: 0.25% No. tested	0.25% dead	0.25% Uncorrected mort.%	0.5% No. tested	0.5% dead	0.5% Uncorrected mort.%	0.75% No. tested	0.75% dead	0.75% Uncorrected mort.%	1.0% No. tested	1.0% dead	1.0% Uncorrected mort.%	1.5% No. tested	1.5% dead	1.5% Uncorrected mort.%	2.0% No. tested	2.0% dead	2.0% Uncorrected mort.%	3.0% No. tested	3.0% dead	3.0% Uncorrected mort.%	4.0% No. tested	4.0% dead	4.0% Uncorrected mort.%	Control No. tested	Control dead	Control Mort. %	DDT LC_{50} (%)
A. quadrimaculatus	J. P. Duret	Mexico	Colony Inst. de Salubridad y Enfermedades Tropicales	-	Sept. & Oct. 1956	79	1	1.3	259	12	4.6				294	41	13.9	249	137	55.0	163	115	70.6							139	1	0.7	1.52*
A. aztecus	"	"	"	-	"	119	15	12.6	277	68	24.5				284	94	33.1	254	113	44.5	283	174	61.5							200	1	0.5	1.60*
A. albimanus	"	"	"	-	"	196	17	8.7	468	175	37.4				591	372	62.9	245	226	92.2	114	108	94.7							197	0	0	0.69*
A. albimanus	J. Blasquez[1]	Venezuela	La Cabrera (Maracay)	DDT sprayed	1957										249	182	73.4				205	188	91.6	198	192	97				120	21	17.5	0.72
A. aquasalis	"	"	"	"	"										205	123	60.0				200	177	88.5	195	179	91.7				206	43	20.9	1.00
A. albitarsis	"	"	El Limon (San Carlos)	sprayed?	"										37	20	54				36	27	75	55	52	94.5				38	6	21.4	1.10
A. gambiae	M. H. Holstein	Belgian Congo	Katana (Near Bukavu)	unsprayed	Jan.-Feb.1957				28	5	17.8				32	22	68.7	35	31	88.5	37	36	97.3	30	30	100	32	32	100	42	1	2.4	0.9
"	"	"	Ruzizi (Near Bukavu)	DDT sprayed	"	27	3	11.1	77	5	6.5				76	13	17.1	77	17	22	75	39	52	51	38	74.5	57	41	71.9	91	1	1.1	1.8
"	J. Armstrong	Zanzibar	various localities Zanzibar island	unsprayed	26/1-2/2/57	40	8	20	60	25	42	70	37	53	60	31	52				40	39	98							50	9	18	0.8
"	R. Elliott & J. Armstrong	Nigeria	Ilaro (southern Nigeria)[4]	BHC sprayed	12-17/11/56	20[5]	1	5	21	5	26				20	17	85				6	5	83				15	15	100	10	0	0	0.6
A. maculipennis	Ungureanu et al.	Romania	Podgorii[2]	unsprayed	20/6-16/8/56										855	239	27.9				882	648	73.5							521	6	1.2	1.4
"	"	"	Podgorii[3]	"	21/9-25/10/56										119	23	19.7				467	221	47.3	119	93	78.2				106	0	0	2.0
"	"	"	Unghemi	sprayed last 8 years (DDT and BHC)	31/7-12/9/56										566	125	22				566	279	49.2							390	8	2.1	2.1
A. maculipennis typicus	J. Hadjinicolaou	Greece	Poros (on Evros river)	sprayed	5-10/9/56				160	56	35				153	101	66				133	92	69.1	162	131	80.8	175	150	85.7	173	18	10.4	1.2
A. sacharovi	"	"	Asterion, Skala (Laconia)	"	14-16/8/56				139	10	7.2				171	15	8.7				313	47	15	210	27	12.7	224	41	18.3	115	2	1.7	122.0*
"	"	"	Anthili, Lamia	"	23-24/8/56				29	1	3.4				76	12	15.7				90	28	31.1	81	44	54.3	78	45	57.7	112	12	10.7	3.0
"	"	"	Nea Komi, Kavalla (Macedonia)	"	13-15/9/56				77	9	11.6				81	22	27.1				87	27	31.0	84	38	45.2	85	58	68.2	72	1	1.4	2.7
A. superpictus	"	"	Triada, Serres (Macedonia)	"	16/9/56				22	17	77.2				18	15	83.3				21	20	95.2	25	25	100	26	26	100	22	4	18.1	<0.5
"	G. Gramiccia et al.	Iran	Sabzevar area	unsprayed	Apr.-Nov.1955	36	1	2.8	369	73	19.8	180	92	51	369	299	81				366	366	100							344	21	6.1	0.72
A. multicolor	"	"	"	"	"				140	92	66				140	130	93				140	130	93										0.5
A. hyrcanus sinensis	P. T. Tseng	Taiwan	Tung-Kang[4]	DDT sprayed	9/9/54				26	6	23.1				26	18	69.2				17	14	82.4							26	0	0	0.81*
"	S. Y. Liu et al.	"	Tung-Kang[4]	DDT sprayed	10-14/3/56	62	15	24.2	61	21	34.4				63	23	36.5				63	52	82.5							63	11	17.5	1.20*
"	S. Y. Liu	"	Wu-Kou-Shui[6]	"	16/2/56 2-3/3/56	95	8	8.4	95	11	11.6				95	31	32.6				69	36	52.2							95	3	3.2	1.97*

* Numerical estimation

1 Information communicated through Environmental Sanitation Division

2 Engorged females

3 Females with fat body

4 Tests made with unengorged females bred from larvae collected in nature or bred from eggs deposited by wild-caught females

5 DDT concentration 0.2%

6 Tests made with engorged females captured in nature in the adult stage

TABLE I. SUSCEPTIBILITY TESTS BY BUSVINE METHOD - DDT RESULTS (continued)

Species	Investigator	Country	Locality or area	Sprayed or unsprayed	Date	DDT concentration in the non-volatile oil: 0.25%			0.5%			0.75%			1.0%			1.5%			2.0%			3.0%			4.0%			Control			DDT LC_{50} (%)
						No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Mort. %	
<u>A. hyrcanus sinensis</u>	S. Y. Liu	Taiwan	Hsin-Chu[1]	DDT sprayed	18, 19 & 29/2/56	102	2	2.0	102	4	3.9				101	15	14.9				119	66	55.5							100	4	4	1.93*
"	"	"	Tung-Kang[1]	"	6-8/3/56	102	5	4.9	101	7	6.9				102	15	14.7				102	44	43.1							102	6	5.9	2.88*
<u>A. hyrcanus</u>[2]	W. T. Chellappah[3]	Singapore	Singapore	unsprayed?	25/7-12/8/55				48	1	2.1				48	3	6.3				72	31	43.1							168	3	1.8	2.3
<u>A. kochi</u>	"	"	"	"	August 1955				18	9	50				42	22	52.4				48	31	64.6							108	7	6.5	0.9
<u>A. vagus</u>	"	"	"	"	23/8-30/8/55				12	7	58.3				42	32	76.1				24	23	95.8							78	15	19.2	0.56
<u>A. sundaicus</u>	S. Sundararaman	Java	Tjilatjap area (S. coast of Java)	unsprayed	Apr.-Dec.1955	454	90	19.8	835	430	51.5	672	410	61.0	922	768	83.4				961	936	97.4							859	44	5.1	0.5
"	"	"	"	sprayed (4 yrs DDT)	April 1956				89	39	43.8				86	61	70.9				82	78	95.1							121	5	4.1	0.6
"	F. Delphin	Burma	Akyab	DDT sprayed past 4 yrs	Nov. 1956	42	15	35.7	30	23	76.6	17	17	100	23	23	100				19	19	100							10	0	0	0.31
<u>A. l. leucosphyrus</u>	W. H. Huehne & Wong	Sarawak	Song (3rd Division)	unsprayed	3-19/12/56				65	46	70.8				68	58	85.3				68	68	100							60	19	31.7	0.41
<u>A. l. balabacensis</u>	T. L. Chang	B.N.Borneo	Keningau	unsprayed	Nov. 1956				47	29	61.7				47	46	97.9				47	46	97.9							47	0	0	0.37
"	"	"	Tenom	dieldrin sprayed	March 1957				26	17	65.4				26	22	84.6				25	24	96							56	0	0	0.36
"	"	"	Tambunan	DDT sprayed	"				31	9	29				33	22	66.6				46	44	95.9							40	1	2.5	0.72

* Numerical estimation

1 Tests made with engorged females captured in nature in the adult stage

2 No attempt was made to establish the sub-specific status of the <u>hyrcanus</u> used in these experiments

3 Information communicated through Environmental Division

TABLE II. SUSCEPTIBILITY TESTS BY BUSVINE METHOD - DIELDRIN RESULTS

Species	Investigator	Country	Locality or area	Sprayed or unsprayed	Date	Dieldrin concentration in the non-volatile oil																								Control			Dieldrin LC_{50} (%)
						0.05%			0.1%			0.2%			0.4%			0.75%			1.0%			2.0%			4.0%						
						No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Uncor-rected mort.%	No. tested	dead	Mort. %	
A. gambiae	M. H. Holstein	Belgian Congo	Katana (near Bukavu)	unsprayed	Jan.-Feb.1957	40	30	75	66	56	85	66	66	100	66	66	100	47	47	100	47	47	100	44	44	100	50	50	100	42	1	2.4	0.015[1]
"	"	"	Ruzizi (near Bukavu)	DDT sprayed	"	68	5	7.3	80	32	40	79	34	43	85	57	67	29	25	86.2	40	38	95	45	45	100	45	45	100				0.22
A. gambiae	J. Armstrong	Zanzibar	various localities Zanzibar island	unsprayed	26/1-2/2/57	50	37	74	concentr. 0.07			44	44	100																40	-	23	0.042
									45	41	91																						
									concentr. 0.1																								
									43	42	98																						
A. sacharovi	J. Hadjinicolaou	Greece	Nea Komi, Kavalla (Macedonia)	sprayed	13-15/9/56				28	2	7.1	61	19	31.1	72	48	66.6													72	1	1.4	0.29
A. maculipennis typicus	"	"	Poros (on Evros river)	"	8-10/9/56				123	50	40.6	113	78	69.0	119	111	95.8													143	16	11.1	0.14
A. superpictus	"	"	Triada, Serres (Macedonia)	"	16/9/56				23	9	39.1	24	18	75	23	20	87													22	4	18.1	0.17
A. hyrcanus	W. T. Chellappah[2]	Singapore	Singapore	unsprayed?	3-20/12/55							48	48	100	48	48	100													96	2	2.1	<0.2
A. kochi	"	"	"	"	13-16/1/56							30	30	100	12	12	100													42	0	0	<0.2
A. vagus	"	"	"	"	22/11-16/12/55							48	42	87.5	48	48	100													96	2	2.1	<0.2
A. l. leucosphyrus	W. H. Huehne & Wong	Sarawak	Song (3rd Division)	"	3-19/12/56	61	56	91.8	62	57	91.9	58	57	98.3																60	19	31.7	0.005
A. l. balabacensis	T. L. Chang	B.N.Borneo	Keningau	unsprayed	Nov. 1956							47	47	100	47	47	100													47	0	0	<0.2
"	"	"	"	"	Feb. 1956				99	99	100	53	53	100	53	53	100													52	0	0	<0.1
"	"	"	Tenom	Dieldrin sprayed	March 1957				48	48	100	50	50	100	49	49	100													56	0	0	<0.1

[1] LC_{50} estimated on mortalities obtained with two concentrations only

[2] Information communicated through Environmental Sanitation Division

TABLE III. SUSCEPTIBILITY TESTS BY BUSVINE METHOD - GAMMA BHC RESULTS

Species	Investigator	Country	Locality or area	Sprayed or unsprayed	Date	Gamma BHC concentration in the non-volatile oil																		Control			Lindane LC_{50} (%)
						0.001%			0.002%			0.005%			0.01%			0.025%			0.04%						
						No. tested	dead	Uncorrected mort.%	No. tested	dead	Uncorrected mort.%	No. tested	dead	Uncorrected mort.%	No. tested	dead	Uncorrected mort.%	No. tested	dead	Uncorrected mort.%	No. tested	dead	Uncorrected mort.%	No. tested	dead	Mort. %	
A. gambiae	J. Armstrong	Zanzibar	various localities Zanzibar island	unsprayed	26/1-2/2/57				40	16	40	40	26	65	40	39	98	40	40	100				39	-	18	0.004
"	R. Elliott & J. Armstrong	Nigeria	Ilaro (S. Nigeria)[1]	BHC sprayed	12-17/11/56	30	2	6.6	29	2	6.8	28	12	42	30	30	100	30	30	100				30	0	0	0.006
A. maculipennis	Ungureanu et al.	Romania	Podgorii	unsprayed	12/7-21/9/56													860	180	20.9	852	564	66.2	494	4	0.8	0.032
"	"	"	Unghemi	sprayed last 8 yrs (DDT & BHC)	31/7-22/8/56													455	151	33.3	456	312	66	360	8	2.2	0.029
A. sacharovi	J. Hadjinicolaou	Greece	Nea Komi, Kavalla (Macedonia)	sprayed	13-15/9/56										27	2	7.4	74	21	28.3	76	44	57.8	72	1	1.4	0.033
A. maculipennis typicus	"	"	Poros (on Evros river)	"	8-10/9/56										123	46	37.3	126	90	71.4	113	109	96.4	143	16	11.1	0.014
A. superpictus	"	"	Triada, Serres (Macedonia)	"	16/9/56										18	9	50	20	17	85	17	15	88.2	22	4	18.1	0.011

[1] Tests made with unengorged females bred from larvae collected in nature or bred from eggs deposited by wild-caught females